

Fig. 1A

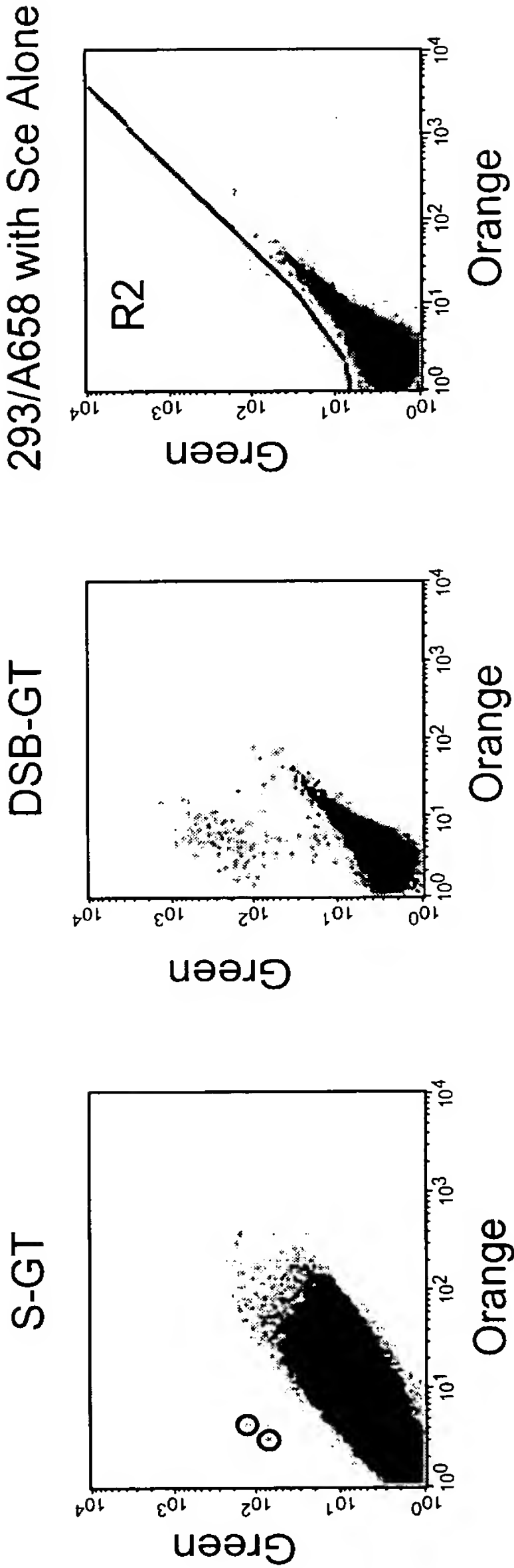


Fig. 1B

Gene Target	<u>1bp insertion</u>		<u>7 bp insertion</u>		<u>35 bp insertion (A658)</u>		<u>35 bp insertion (A658)</u>		<u>66 bp insertion (QQR8)</u>		Fold Stimulation By Sce Induced DSB
	No		No		No		Yes		Yes		
Sce											
Events/ 10 ⁶ cells	29.5 +/- 13.0		1.5 +/- 1.1		0.71 +/- 0.27		1600 +/- 500		1900 +/- 250		>2000
Rate	2.9 x 10 ⁻⁵		1.5 x 10 ⁻⁶		7.1 x 10 ⁻⁷		1.6 x 10 ⁻³		1.9 x 10 ⁻³		

Fig. 1C

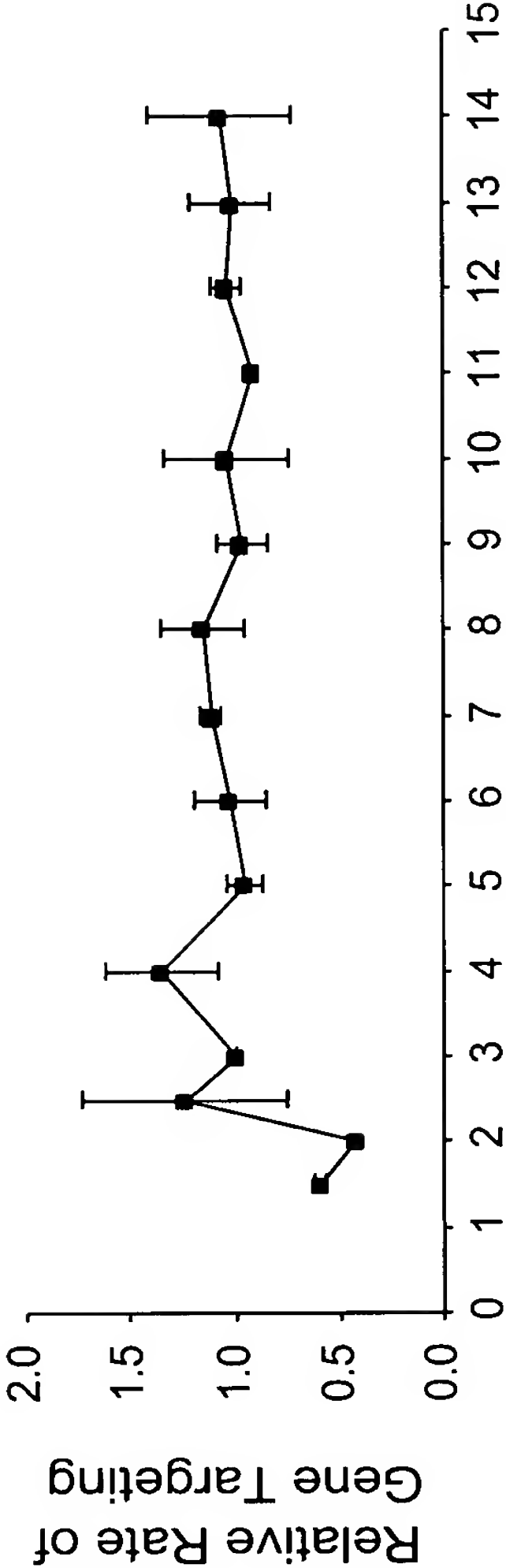


Fig. 1D

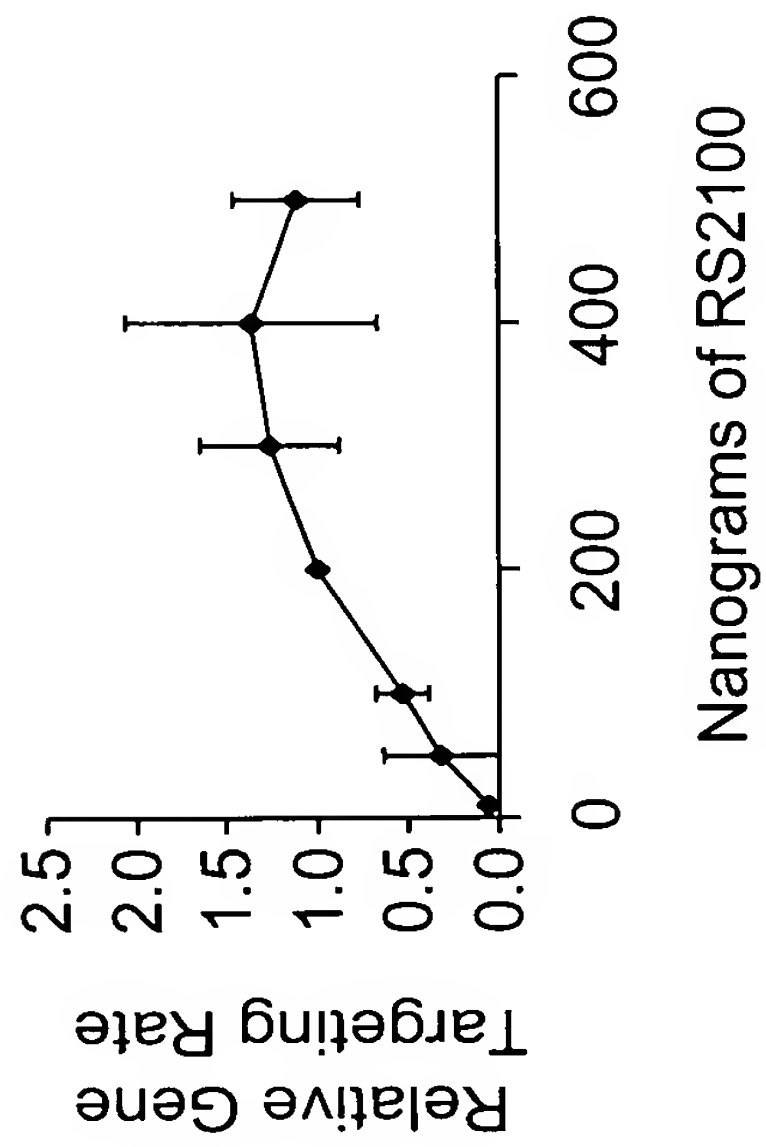


Fig. 2A

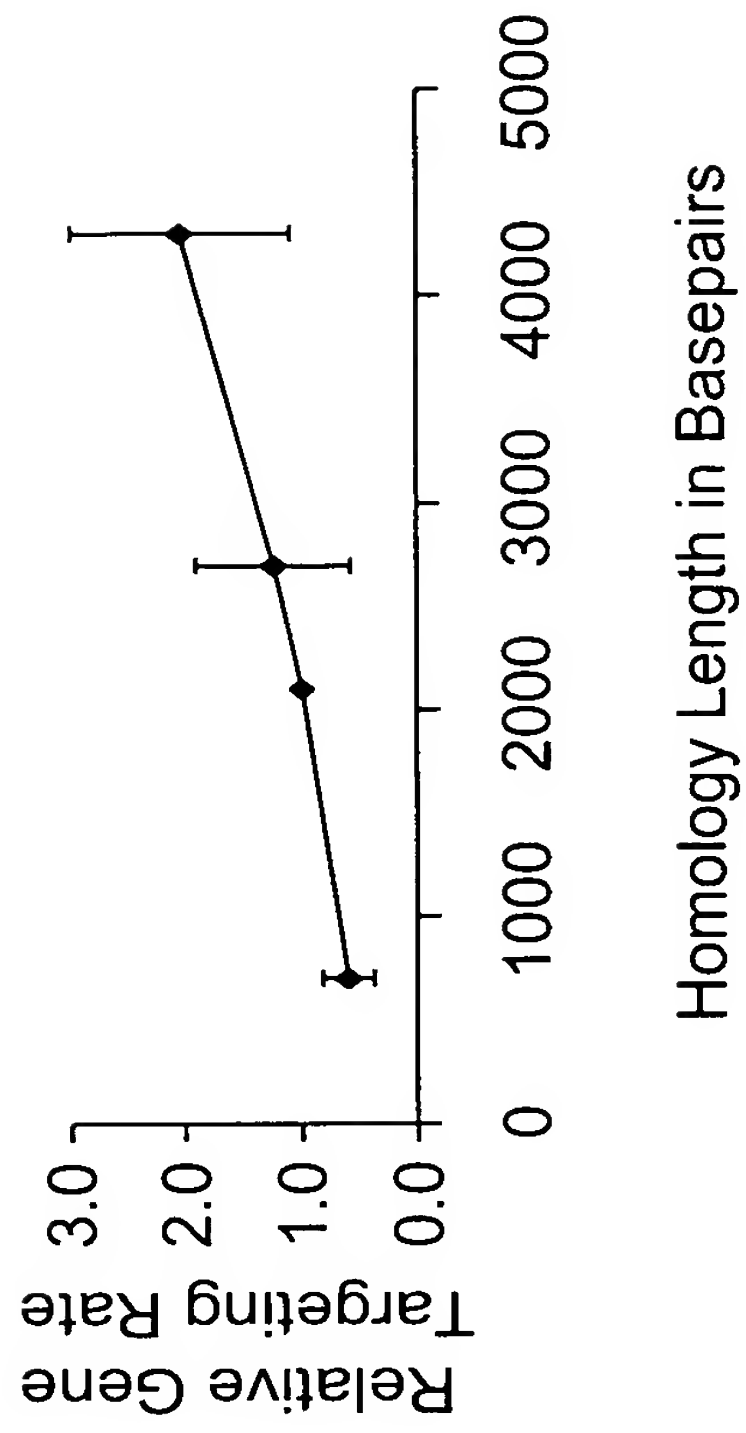


Fig. 2B

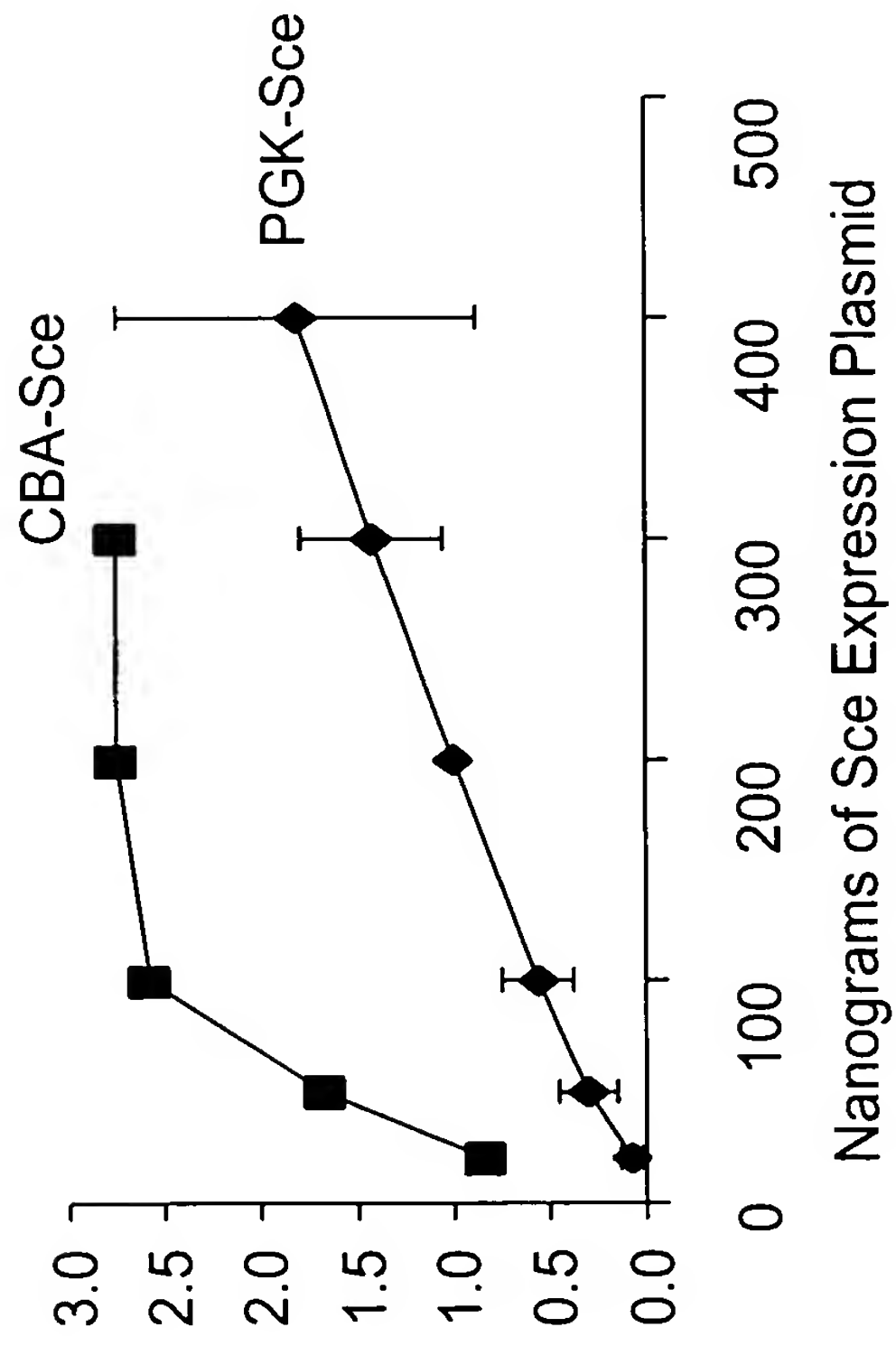


Fig. 2C

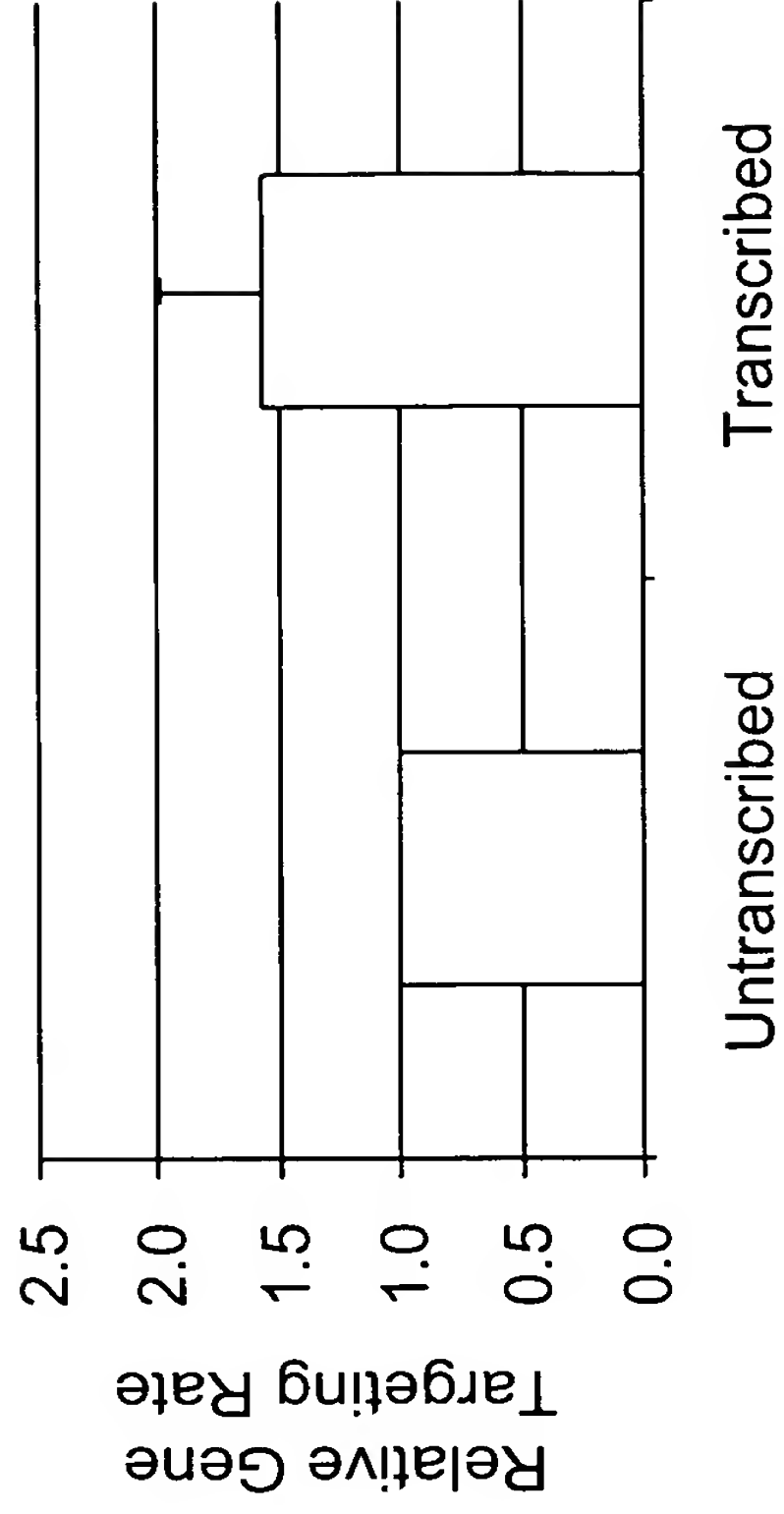


Fig. 2D

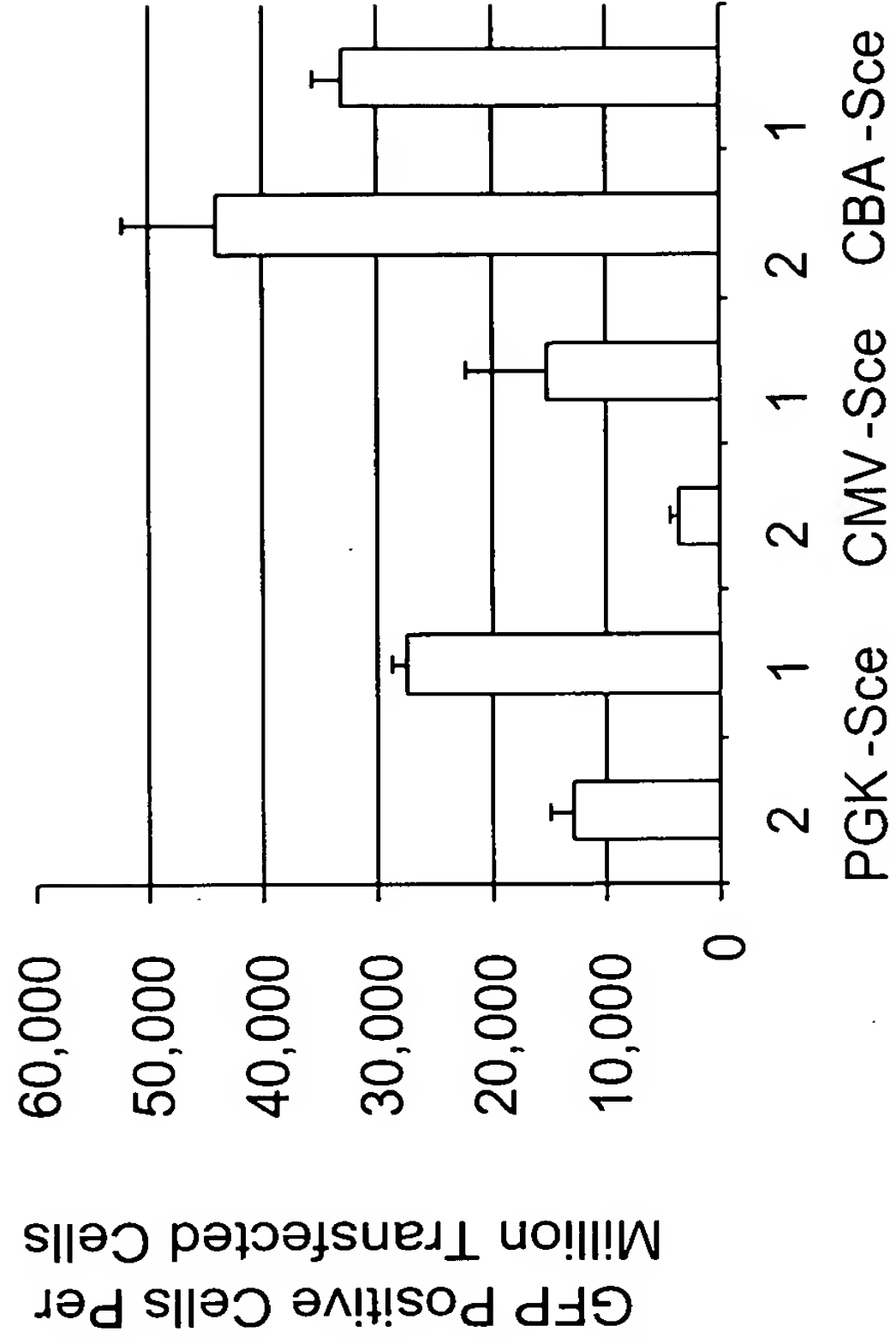


Fig. 2E

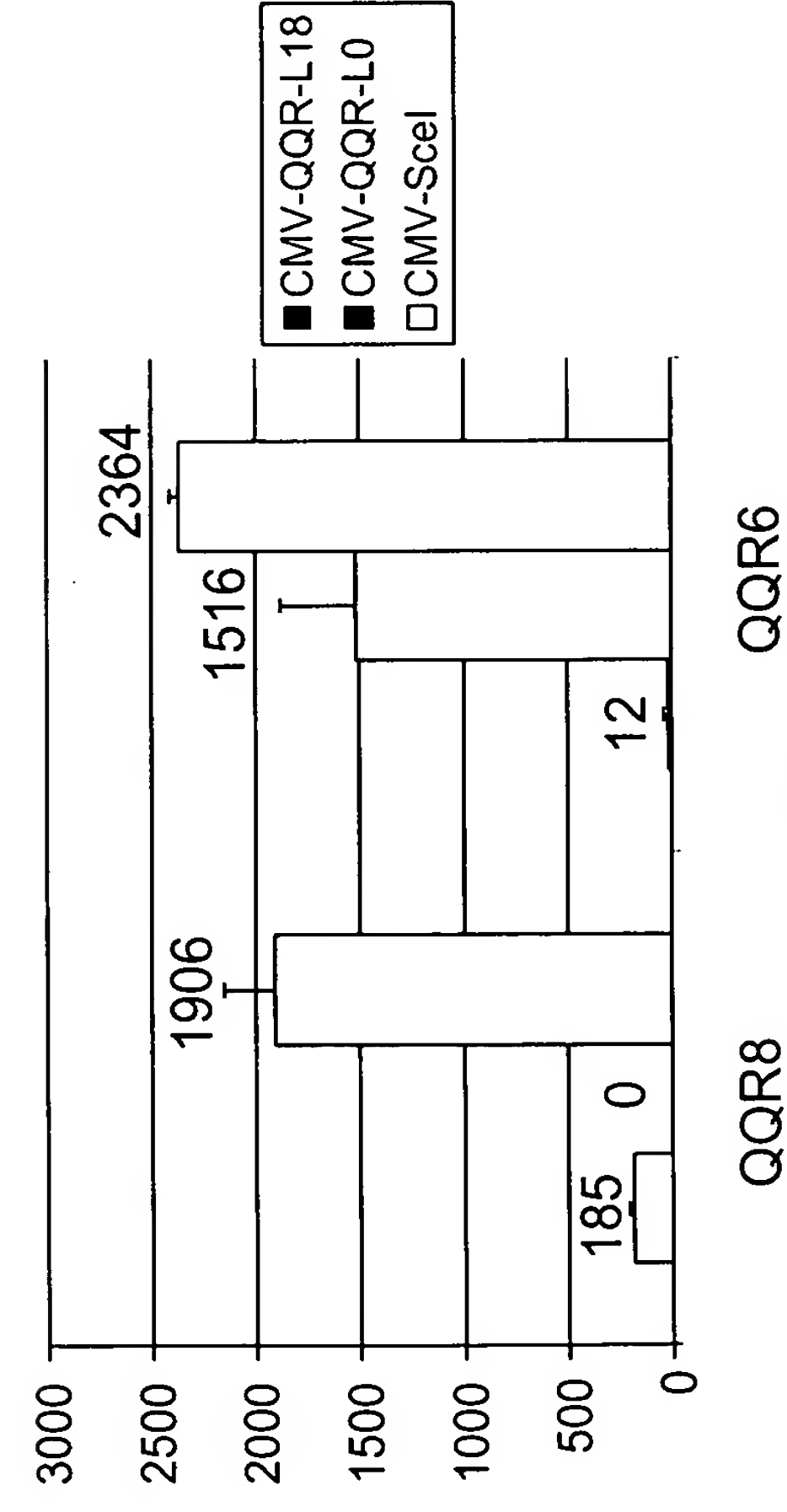


Fig. 3B

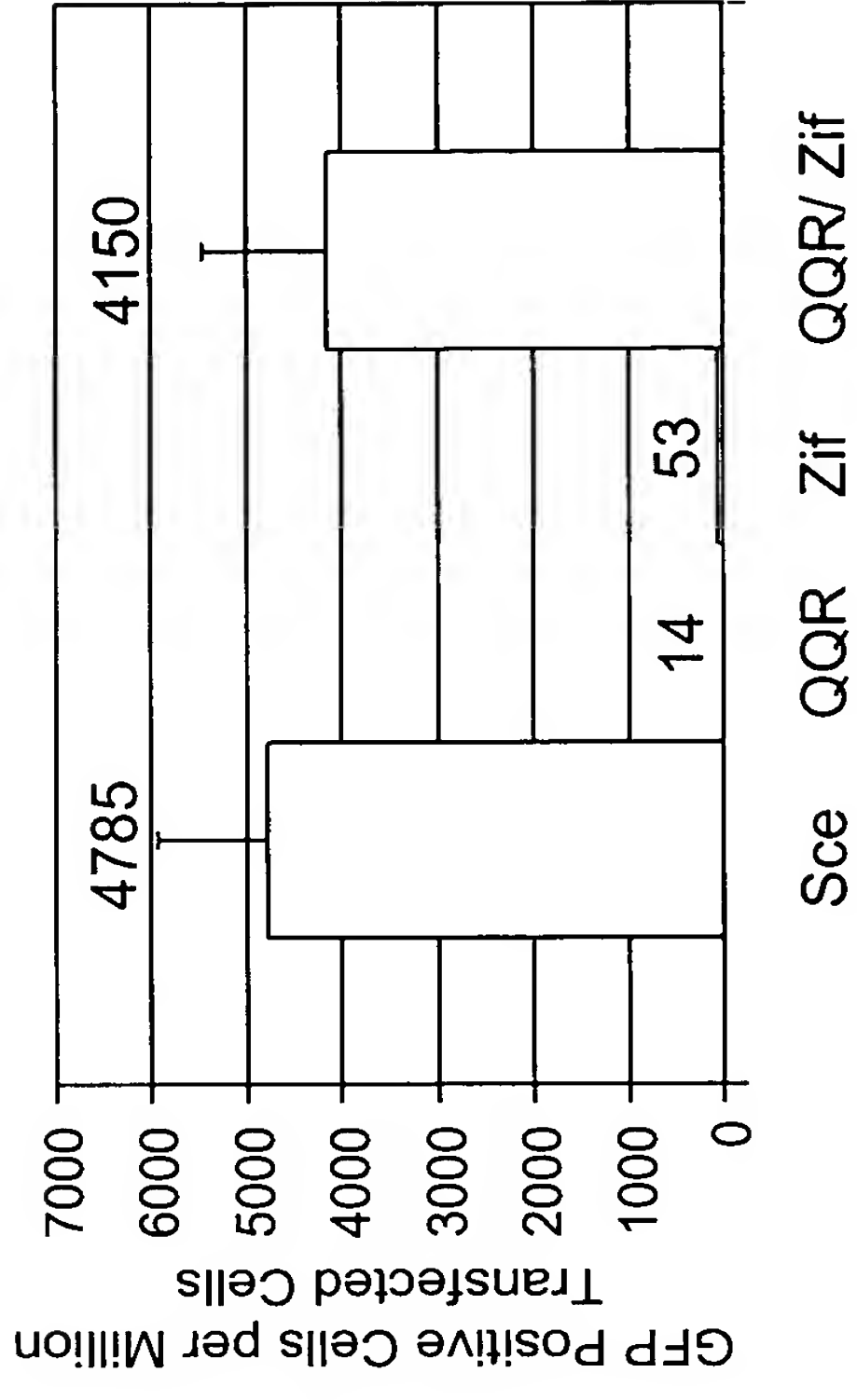


Fig. 3C

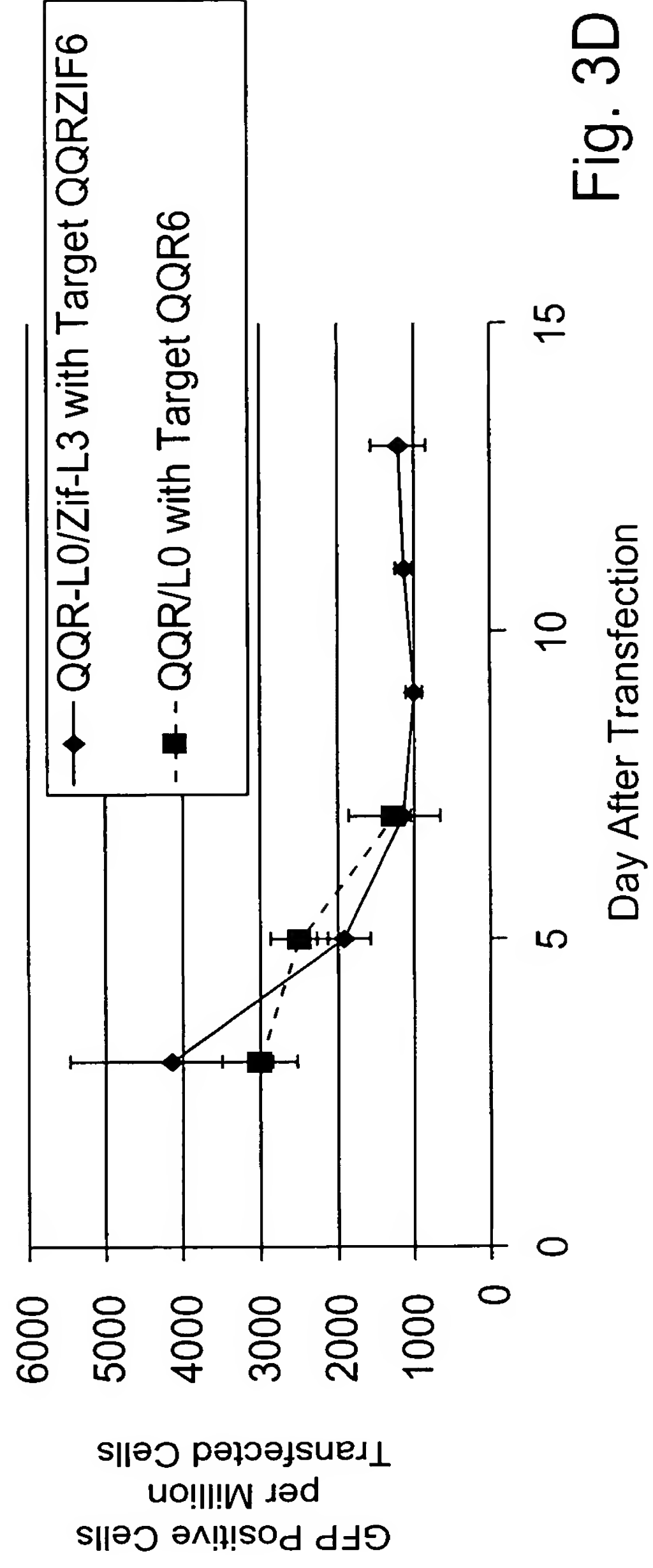


Fig. 3D

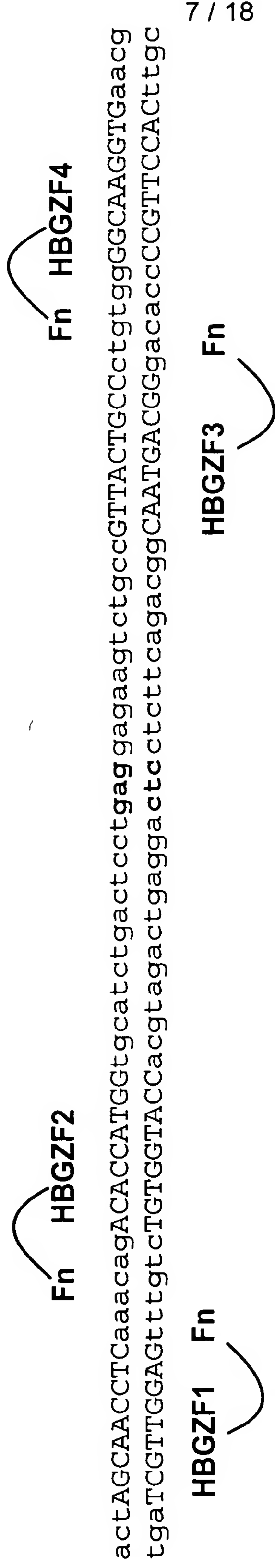


Fig. 4

Design of HBGZF1

Target Binding Site: 5' GAG GTT GCT 3'
F3 F2 F1

-1 1 2 3 4 5 6
B3 B2 B1

Finger 1:	GCT	Q	S	S	D	L	T	R*
		T	S	G	E	L	V	R
Finger 2:	GTT	T	S	G	A	L	T	R*
		T	S	G	S	L	T	R
Finger 3:	GAG	R	S	D	N	L	T	R*
		R	S	D	N	L	V	R

Structure of CMV - HBGZF1

CMV	ATG - NLS - FLAG - Finger 1(QSSDLTR) - Finger 2(TSGALTR) - Finger 3(RSDNLTR) - Fn
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Fig. 5

Gene Targeting with HBGZF1

Target: GFP-Stop-AGCAACCTCtctagaCGTGGGCG-SceSite-GFP
TCGTTGGAGagatctCGACCCGC

HBGZF1 Fn

Fn Zif268

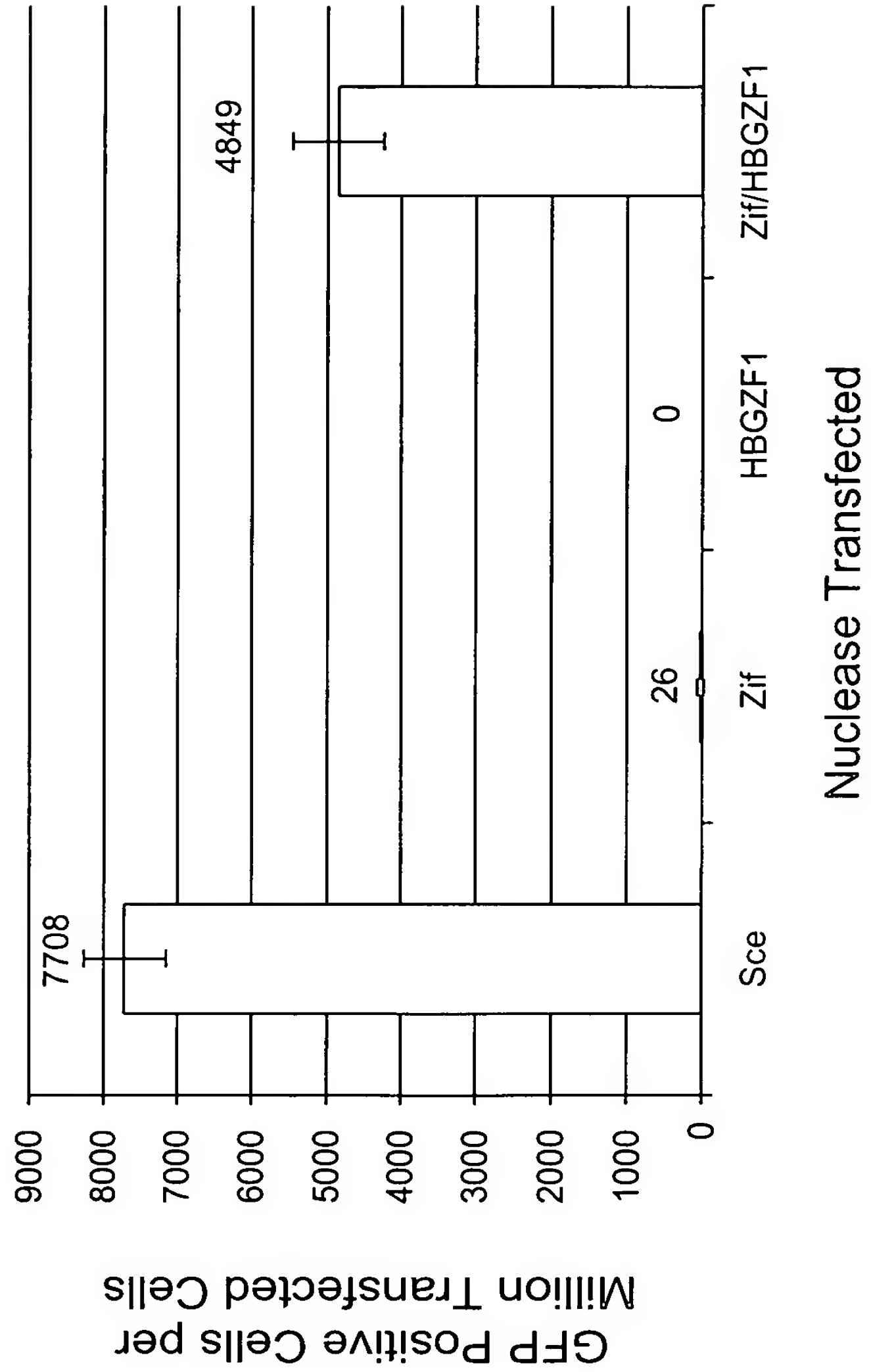


Fig. 6

Design of HBGZF4

Target Binding Site: 5' GGC AAG GTG 3'
F3 F2 F1

-1 1 2 3 4 5 6
B3 B2 B1

Finger 1: GTG R S D A L T R*
R S N S L T R

Finger 2: AAG R S D T L S N
R K D N L K N
R S S N L T Q*

Finger 3: GGC D R S H L A R*
E S N H L T R
E R S K L A R
D P G H L V R

Structure of CMV-HBGZF1

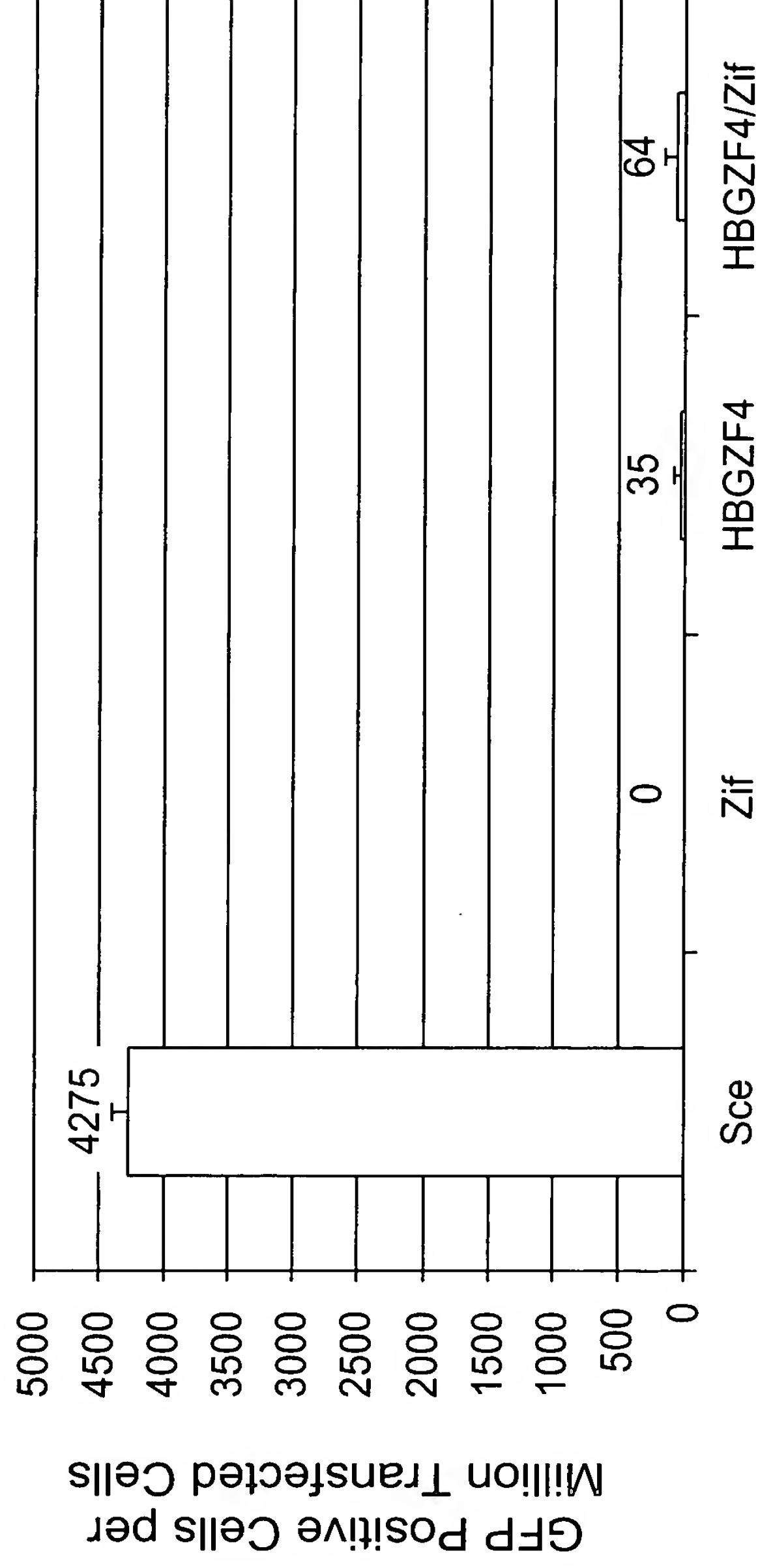
CMV ATG-NLS-FLAG-Finger 1(RSDALTR)-Finger2(RSSNLTQ)-Finger3(DRSHLAR)-Fn

Fig. 7

Gene Targeting with HBGZF4

Fn ZIF268

Target: GFP-Stop-CACCTTGCCctctagaGCGTGGCGG-SceSiteGFP
GTGGAACGGagatctGCACCCGC

HBGF4
Fn

Nuclease Transfected

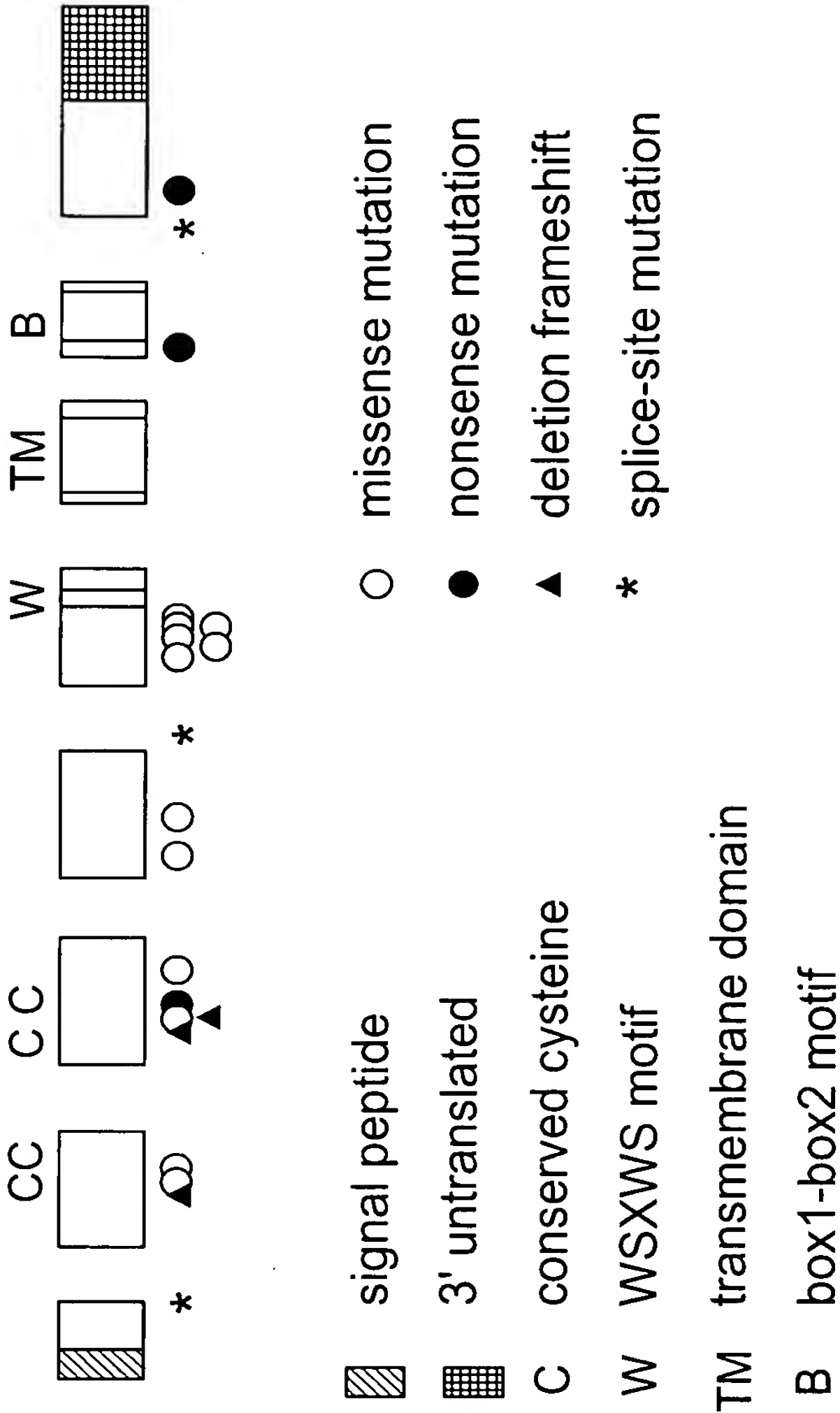


Fig. 9A

Exon 5

5' gaacaatcagtggttataagacataaagttctccttgccctagtggtggatgggcagaaacgctacacgtttcgtgtt
3' cttgttagtcacctaataatctgtattcaagaggaaacggatcacacctaccgtctttgcgatgtgcaaagcacaa

WSXWS motif

HCGCZF2

cggagccgctttaaccactctgtggaagtgtcagcat tggagtggaatgga gccacccaa tccactgggggagca
gcctcggcgaaattgggtgagacaccttcacgagtcgtaacctcacttacctcggtgggttaggtgacccccctcgt

HCGCZF1

atacttcaaaagag 3'
tatgaagttttctg 5'

Fig. 9B

Design of HCGCZF2

Target Binding Site: 5' GGG GGA GCA 3'

F3 F2 F1

-1 1 2 3 4 5 6
B3 B2 B1

Finger 1: GCA

Q	S	G	S	L	T	R
Q	S	G	D	L	T	R
Q	S	G	D	L	T	R
Q	S	N	D	L	T	R*

Finger 2: GGA

Q	S	G	H	L	Q	K
Q	R	A	H	L	E	R
Q	S	S	H	L	T	R*

Finger 3: GGG

R	S	D	H	L	A	R
R	S	D	H	L	T	R
R	S	S	H	L	T	R*

Structure of CMV-HCGCZF2

CMV

ATG-NLS-FLAG-Finger 1(QSNDLTR)-Finger2(QSSHLTR)-Finger3(RSSHLTR)-Fn

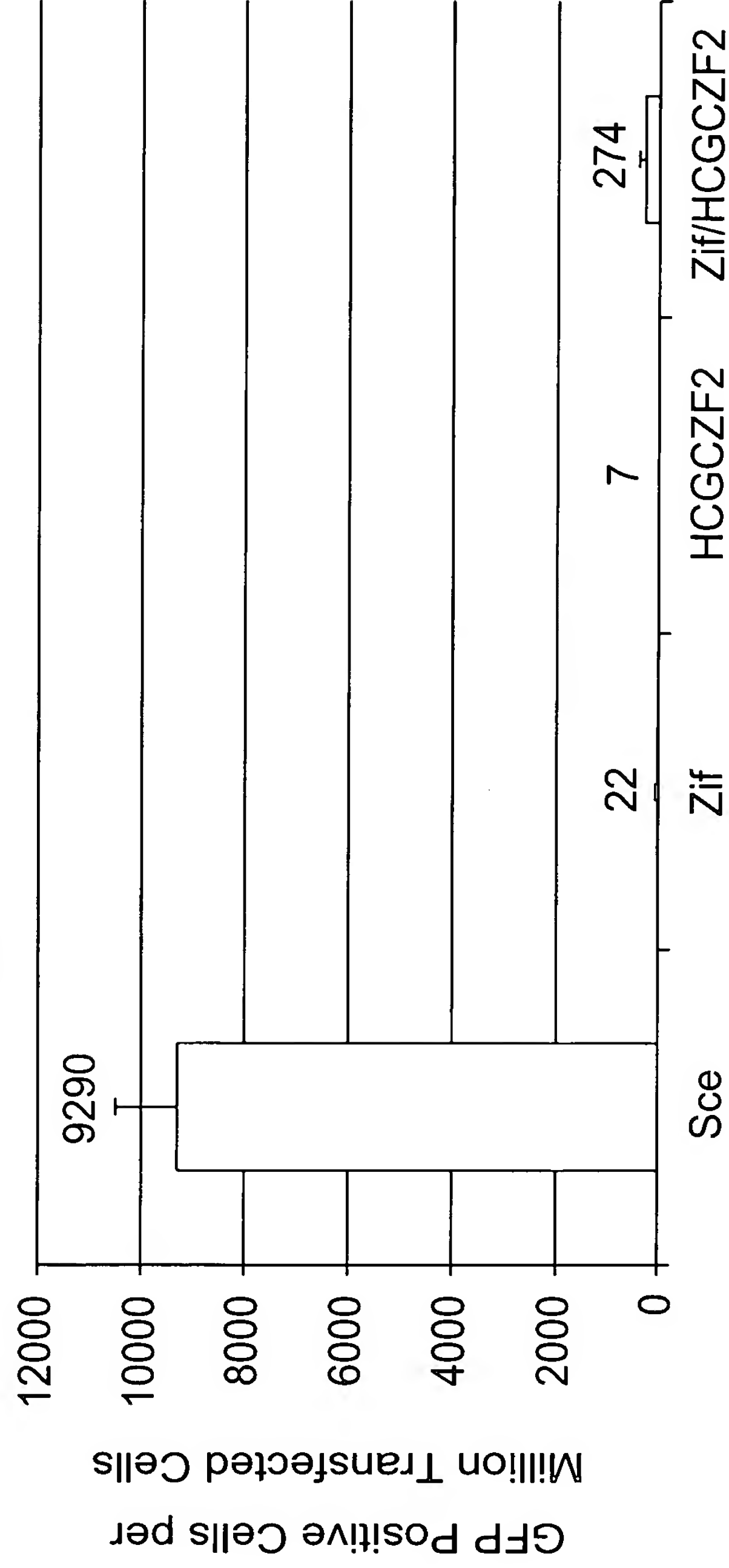
Fig. 10

Gene Targeting with HCGCZF2

Fn ZIF268

Target: GFP-Stop-TGCTCCCCCtctagaCGTGGGCG-SceSite-GFP
ACGAGGGGagatctCGACCCGC

HCGCZF2 Fn



Nuclease Transfected

Fig. 11

Fn  **GFPCN2**

5'acC atC ttc ttcaag Gac Gac Ggc aac stop-Sce site tac
3'tgG taG aaG aag ttc Cgc Ctg Ccg ttc


GFPCN1  **Fn**

Fig. 12A

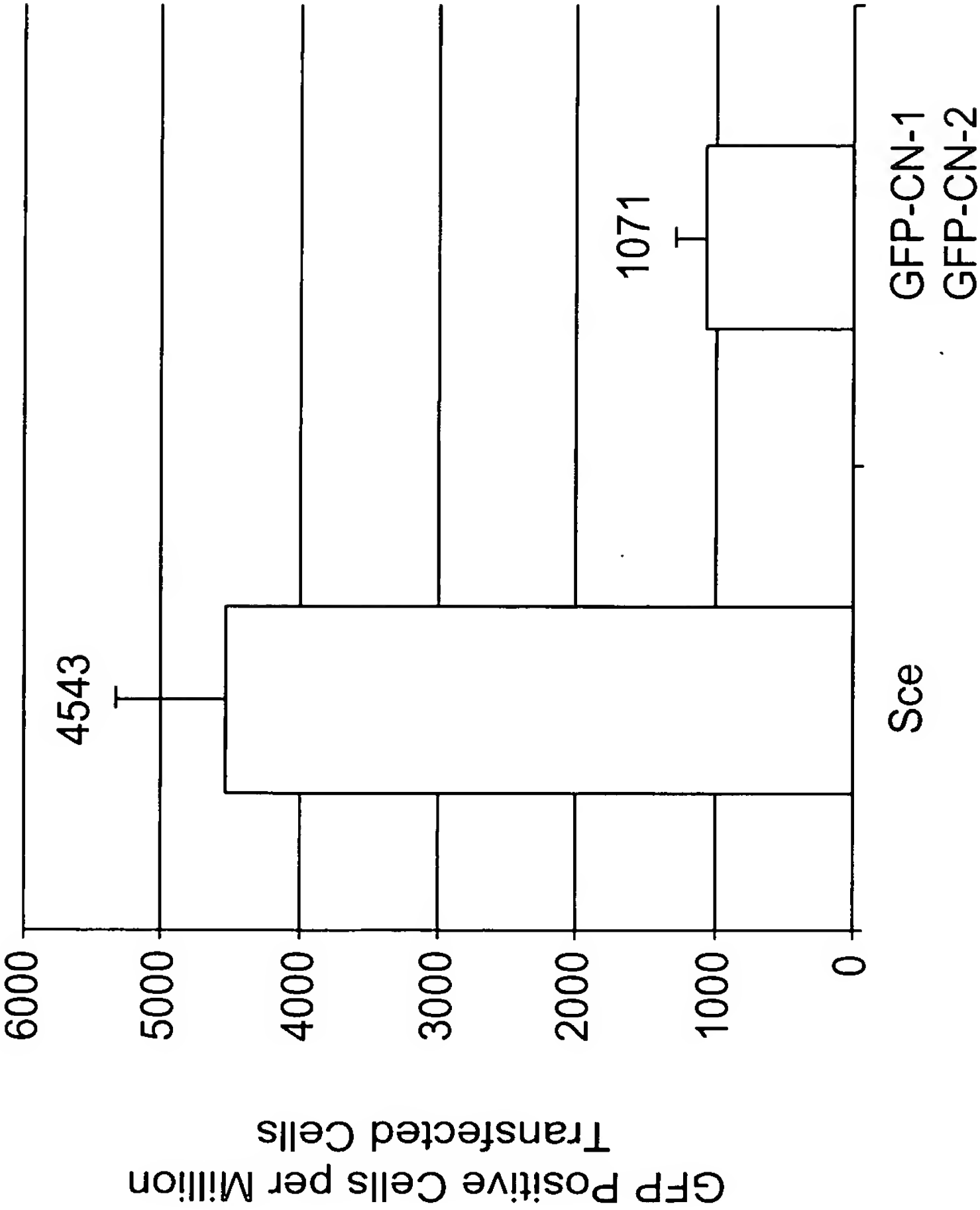


Fig. 12B

Fn  **CD8ZF2**

bp 441 5' acc gg**C**gc**C**ca**C** catcgc Gtc**G** ca**G**cc ctg3' bp 471
tgg cc**G** cg**G**gt**G** gtagcg CagCgt**C**gg gac

CD8ZF1  **Fn**

Fig. 13A

Cell Line 293/1104	CMV/CBA	GFP*	IRES	CD8	95% CD8 Positive
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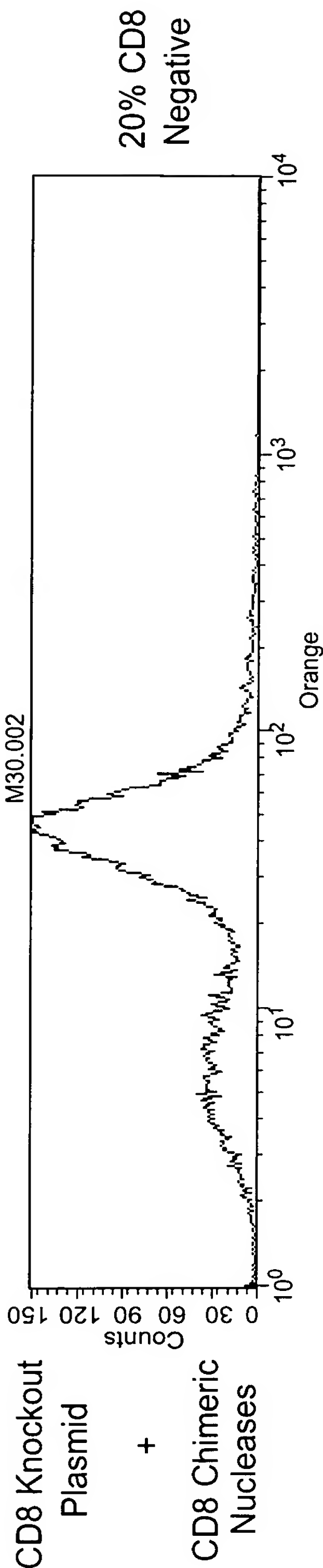
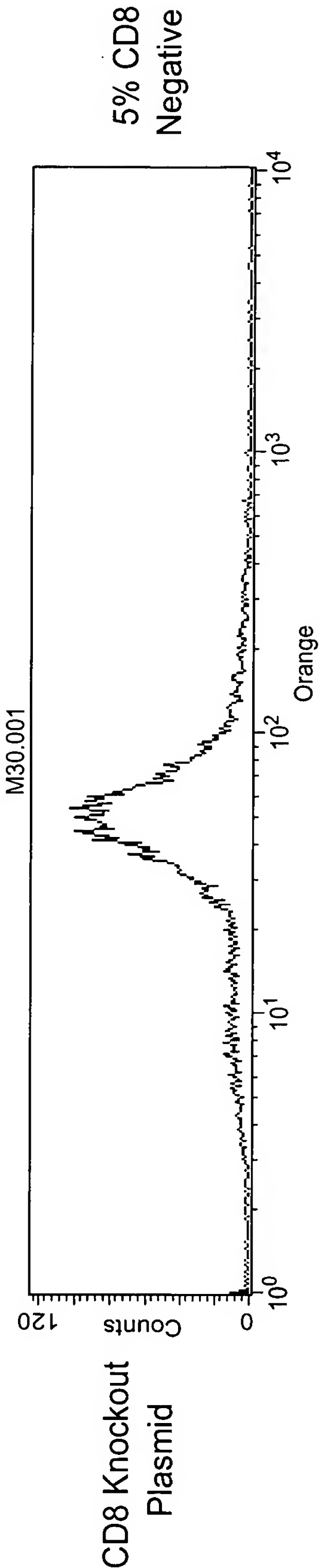


Fig. 13B